A comparison of oral artesunate and artemether antimalarial bioactivities in acute falciparum malaria

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Aims Artesunate and artemether are the two most widely used artemisinin derivatives in the treatment of uncomplicated *Plasmodium falciparum* malaria, but there is little information on their comparative pharmacokinetics. The aim of this study was to examine the relative oral antimalarial bioavailability and pharmacokinetics of the two derivatives.

Methods The pharmacokinetic properties of oral artesunate and artemether (4 mg kg^{-1}) were compared in a randomized cross-over study of 14 adult patients in western Thailand with acute uncomplicated *Plasmodium falciparum* malaria. Antimalarial activity was compared using a previously validated, sensitive bioassay.

Results Despite a 29% lower molar dose, oral artesunate administration resulted in significantly larger mean area under the plasma antimalarial activity time curve and median maximum plasma antimalarial activity than after oral artemether ($P \leq 0.02$). The mean (95% CI) oral antimalarial bioavailability of artemether, relative to oral artesunate, corrected for molar dose was 58 (40–76)%. The mean (95% CI) relative antimalarial bioavailability of artemether was lower on the first day of treatment, 31 (17–100)%, compared to the second day, 72 (44–118)% ($P = 0.018$). *In vivo* parasite clearance and time above the *in vitro* IC_{90} were similar for the two drugs, despite considerable differences in C_{max} and AUC.

Conclusions The oral antimalarial bioavailability following artemether was significantly lower than that after artesunate. Artemether oral antimalarial bioavailability is reduced in acute malaria.

Keywords: artemether, artesunate, bioassay, bioavailability, combination, malaria, pharmacokinetics, *Plasmodium falciparum*, Thailand

Introduction

Artesunate and artemether are the two most widely used oral artemisinin derivatives. They are being used increasingly in South-east Asia and other areas of the world where multidrug resistant *Plasmodium falciparum* malaria is prevalent [1, 2]. Both are prescribed either on their own, or, increasingly, as part of combination treatment, with the intention of providing mutual protection against resistance and enhanced efficacy. Artesunate combined with

mefloquine is now the standard recommended treatment for multidrug resistant falciparum malaria on the western border of Thailand [3]. Artemether-lumefantrine is a new, fixed dose ratio, combination which is well tolerated, and equally effective compared with the artesunate-mefloquine combination [4]. Artesunate is the water soluble sodium hemisuccinyl ester, whilst artemether is the lipid soluble methyl ether of dihydroartemisinin. Both artesunate and artemether are metabolized *in vivo* to the highly active antimalarial metabolite, dihydroartemisinin (DHA) [5, 6]. Oral DHA itself is also effective in the treatment of uncomplicated malaria [7].

The choice of which oral artemisinin derivative to use in different clinical situations has been largely empirical. Recent comparative clinical trials have suggested that

artesunate and artemether are equally effective in uncomplicated falciparum malaria, used either alone or in combination with mefloquine [8, 9]. The pharmaco-kinetics of artesunate and artemether have been studied individually in patients with uncomplicated malaria [5, 10–17]. However, there have been no direct comparative, cross-over pharmacokinetic assessments of the two derivatives.

In terms of current *in vitro* IC_{90} in western Thailand, artemether, artesunate and dihydroartemisinin have relative antimalarial activities of approximately 0.3, 0.7 and 1.0, respectively, although accurate comparisons are complicated by poor solubility and the hydrolysis of artemether and particularly artesunate to DHA in malaria culture media [18]. Chemical methods for the assay of these drugs are difficult, expensive, and still have a limit of accurate detection above the range of concentrations which provide significant antimalarial effect. Bioassay provides an alternative and more sensitive measure, which is a summation of the antimalarial activities of the parent drugs and their active metabolites [15, 16]. We have used a previously validated, sensitive bioassay to compare the antimalarial bioavailability and disposition of oral artesunate and artemether in acute uncomplicated falciparum malaria.

Methods

Patients

This study was conducted in Sangklaburi Hospital, western Thailand. Non-pregnant febrile adults (>14 years) with uncomplicated acute *P. falciparum* malaria were included in the study provided that they gave fully informed verbal consent and had not received previous treatment during their current illness with an artemisinin derivative. Patients were enrolled only if there was a clear history of no previous antimalarial drug treatment. Previous quinine administration was checked using a previously validated urine dipstick screening method [19]. The study was approved by the ethics and scientific review subcommittee of the Royal Thai Government Ministry of Public Health.

Clinical procedures

On admission the patients were weighed, a full clinical examination was conducted, and haematocrit, serum urea and electrolytes, creatinine, liver function tests and plasma glucose and lactate were measured. Thick and thin blood smears were taken and quantitative parasite counts recorded.

Drug and sampling regimes

Patients were randomized in blocks of 10 initially to receive 4 mg kg^{-1} body weight of either:

(a) artesunate tablets (Guilin No. 1 factory, Guangxi, Peoples Republic of China (PRC))

(b) artemether capsules (Kunming Pharmaceutical Factory, Kunming, PRC).

Artesunate tablets were cut and weighed and the powder from artemether capsules removed, weighed and replaced within the capsule to provide the approximate body weight-adjusted dose, which was taken immediately by the patient. For the second dose, 24 h later, the opposite treatment was administered, i.e. if patients received artesunate first, they received artemether on the second day and *vice-versa*. On the third day mefloquine (Lariam®, Roche) 15 mg kg^{-1} was given, followed 12 h later by 10 mg kg^{-1} to complete anti-malarial treatment. Heparinized blood samples (2 ml) were taken through an indwelling forearm vein catheter at 0, 15, 30, 45, 60, 90 and 120 min and then 3, 4, 6, 8, 12, 18 and 24 h following the administration of both artesunate and artemether. Vital signs were recorded 4 hourly and haematocrit and parasitaemia were measured 6 hourly until parasite clearance (defined as the first negative thick film after counting 200 white cells).

Drug assay

Immediately after they were taken, blood samples were centrifuged and the plasma was stored at $-50°$ C for up to 1 month and then at $-80°$ C until assay after 48 months. Antimalarial activity in plasma was measured as described previously by an *in vitro P. falciparum* bioassay (using the W 2 chloroquine-resistant clone) in which antimalarial activity is expressed as dihydroartemisinin equivalents [16]. The bioassay lower limit of quantification was 8.8 nmol l^{-1} and interassay coefficients of variation were 9–13% for DHA concentrations in the range from 18 to 176 nmol l^{-1}. All bioassays were carried out in duplicate and serial dilutions were used for quantification above a concentration of 352 nmol l^{-1} DHA equivalents.

Pharmacokinetic and statistical analysis

A noncompartmental model was fitted to the plasma concentration time data and standard pharmacokinetic parameters derived using WinNonlin™ (Model no. 200; Version 1.1, SCI, Cary, NC, USA [20]). The terminal elimination phase rate constant (λ_z) was calculated by log-linear regression. Area under the curve (AUC(0,∞)) was calculated using the linear trapezoid rule with

log-linear extrapolation to infinity. Oral clearance per fraction of drug absorbed (CL/F) was calculated as dose/AUC(0,∞), volume of distribution (V_z/F) as dose/($\lambda_z \times$ AUC(0,∞)), and mean residence time (MRT) as AUMC(0,∞)/AUC(0,∞) (AUMC(0,∞) is the area under the first moment curve) [20]. Relative antimalarial bioavailability was calculated from the equation:

$$\frac{\text{AUC}_{\text{artemether: }0,\infty} \times \text{dose}_{\text{artesunate}}}{\text{AUC}_{\text{artesunate: }0,\infty} \times \text{dose}_{\text{artemether}}}$$

The terminal elimination phase rate constant λ_z was calculated from a minimum of three data points. One artesunate data set was excluded as only two data points were available for calculating λ_z. Visual inspection of the individual log-linear plots of drug levels against time with the fitted regression equations showed good agreement between the observed and predicted drug levels. r^2 (adjusted for number of data points) for the regression equation used to calculate λ_z was >0.8 in 86% of the cases.

For comparison, the dose and plasma concentrations of the study drugs were expressed in molar terms. The molecular weight of artesunate (384.4) is 29% greater than that for artemether (298.4). A 50 mg artesunate tablet contains 130 μmol and a 40 mg artemether capsule 134 μmol of the respective drug. A 4 mg kg^{-1} body weight dose of artesunate is equivalent to 10 404 nmol kg^{-1} and 4 mg kg^{-1} of artemether is equivalent to 13 404 nmol kg^{-1}.

Parametric (Student's *t*) and nonparametric (Wilcoxon signed-rank and Mann–Whitney) statistical tests were used for paired and unpaired comparisons. Correlations were assessed using linear regression. Analysis was performed using SPSS 8.0 (SPSS Inc., Chicago).

Results

Clinical and laboratory findings

Fourteen adult patients (aged 18–38 years, 7 male, 7 female) with uncomplicated falciparum malaria [21] were enrolled in the study. The patients presented having been ill for a median (range) of 3 days (1–7) with fever, headache, nausea and vomiting. Mean (95% CI; range) body weight was 50.0 kg (46.4, 53.6; 37.0–60.0). The geometric mean (range) admission parasitaemia was 27 194 (720–254 214) μl^{-1} with a median (range) admission plasma lactate of 1.75 (0.8–3.6) mmol l^{-1}. The mean (95% CI) actual doses ingested were 4.03 mg kg^{-1} (3.86, 4.19) for both artesunate and artemether. In molar terms the mean (95% CI) actual dose ingested was greater for artemether, 13578 nmol kg^{-1} (13071, 14085),

than for artesunate, 10539 nmol kg^{-1} (10146, 10932) ($P < 0.0001$). All patients made a rapid and uncomplicated recovery. The median (range) parasite clearance time was 32 h (12–65). There were no significant differences in clinical or laboratory features between those patients who received artesunate or artemether first ($P > 0.13$).

One patient was found to have received quinine and one chloroquine, before the study, but the bioassay method controlled for these potential confounders by calibrating the time zero plasma sample as zero DHA equivalents. In these two cases the antimalarial activities from quinine and chloroquine (half-life 16–18 h and 30–60 days, respectively [2]) were assumed not to have changed over the 4–6 h after artesunate and artemether dosing. The only other oral drugs taken by the patients during the study were: paracetamol (9 patients), dimenhydrinate (5), diazepam (1), metoclopramide (1), diphenhydramine (1), domperidone (1) and ferrous sulphate (1). None of these is known to interact with either artesunate or artemether. No study drug adverse effects were noted.

Absorption

Oral artesunate and artemether were both absorbed rapidly, reaching peak concentrations in a median of 1.5 and 2.0 h, respectively (Table 1, Figure 1). The median peak plasma antimalarial concentration ($C_{\max}$) was significantly higher after artesunate than after artemether administration. The corresponding median (range) $C_{\max}$ corrected for molar dose/body weight was 0.78 (0.31–2.37) nmol l^{-1} nmol^{-1} kg^{-1} after artesunate and 0.27 (0.05–0.35) nmol l^{-1} nmol^{-1} kg^{-1} after artemether ($P = 0.004$).

Disposition

Despite the 29% higher molar dose, artemether administration gave significantly lower estimates of AUC(0,∞), and thus greater apparent clearance, larger apparent volumes of distribution and longer mean residence time values than after artesunate administration (Table 1). The mean (95% CI) relative antimalarial activity bioavailability of artemether compared with artesunate, corrected for molar equivalent doses, was 58 (40–76)%. Antimalarial activity 24 h after the first dose was negligible in comparison with that within the first 6 h after the subsequent dose (see Figures 1, 2 and 3), confirming that there was no requirement for a longer washout period between treatments. There were no significant relationships between any of the derived pharmacokinetic variables and admission clinical and laboratory measurements ($P > 0.02$).

Table 1 Median (range) and mean (95% CI)[a,b] pharmacokinetic variables for oral administration of artesunate and artemether. Bioassay in DHA equivalents, except molar doses.

Variable	Artesunate[c]	Artemether[c]	P
Molar dose (nmol kg^{-1})[a]	10 539 (10 146–10 932)	13 578 (13 071–14 085)	<0.0001
t_{lag} (h)	0.25 (0.25)	0.25 (0.25–1.50)	0.06
t_{max} (h)	1.50 (0.50–4.00)	2.0 (0.75–6.00)	0.57
C_{max} (nmol l^{-1})	8088 (4568–24 633)	3454 (909–4663)	0.006
$t_{1/2}$ (h)	1.31 (0.55–4.71)	2.98 (1.22–5.44)	0.028
λ_z (h^{-1})	0.574 (0.147–1.263)	0.236 (0.127–0.568)	0.009
AUC(0,∞) (nmol l^{-1} h)[b]	18 867 (16 146–21 588)	11 129 (9007–13 251)	0.020
V_z/F (l kg^{-1})	1.31 (0.34–5.59)	4.37 (1.73–13.85)	0.004
CL/F (l kg^{-1} h^{-1})	0.65 (0.27–1.57)	1.28 (0.69–2.55)	0.004
MRT (h)	2.52 (1.18–5.11)	3.97 (0.58–6.31)	0.019

Abbreviations: C_{max} (maximum observed concentration); t_{max} (observed time to C_{max}); t_{lag} (absorption lag time); $t_{1/2}$ (elimination half-life); λ_z (elimination rate constant); V_z/F (total apparent volume of distribution kg^{-1} body weight); MRT (mean residence time); CL/F (clearance kg^{-1} body weight); AUC(0,∞) (area under the antimalarial activity – time curve extrapolated to infinity).
[a]Difference between the means (95% CI) = 3039 (2896, 3182) nmol kg^{-1}; [b]Difference between the means (95% CI) = 7738 (3568, 11908) h nmol l^{-1}; [c]To convert DHA equivalents nmol l^{-1} to ng ml^{-1} divide by 3.517.
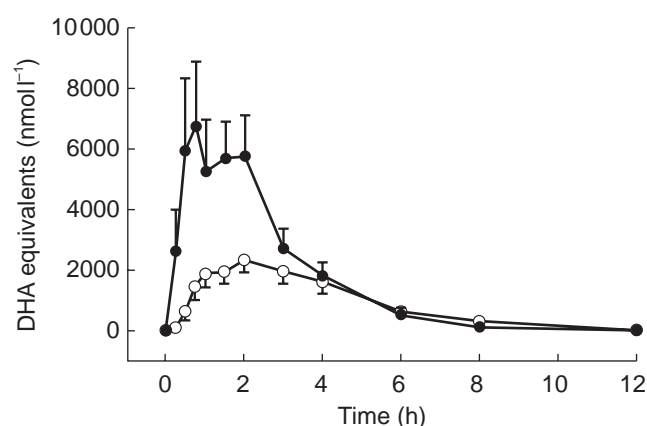
Figure 1 Mean antimalarial activity ($\pm$s.e. mean) in DHA equivalents for oral administration of artesunate ($\bullet$) and artemether ($\circ$) during the first 12 h after drug administration. Days 1 and 2 combined.
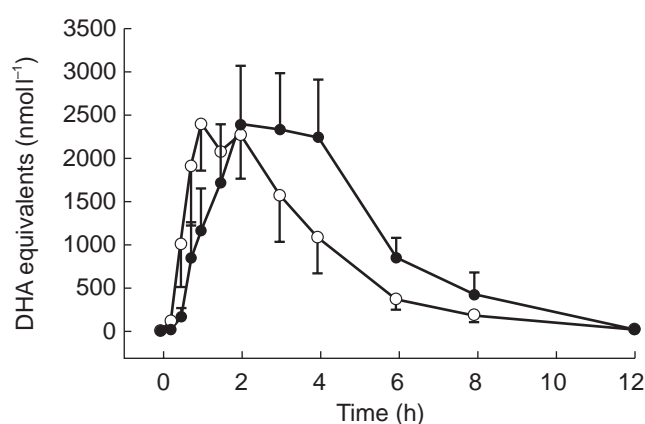
Figure 3 Mean antimalarial activity ($\pm$s.e. mean) in DHA equivalents for oral administration of artemether, on the first ($\circ$) and second day ($\bullet$), during the first 12 h after drug administration.
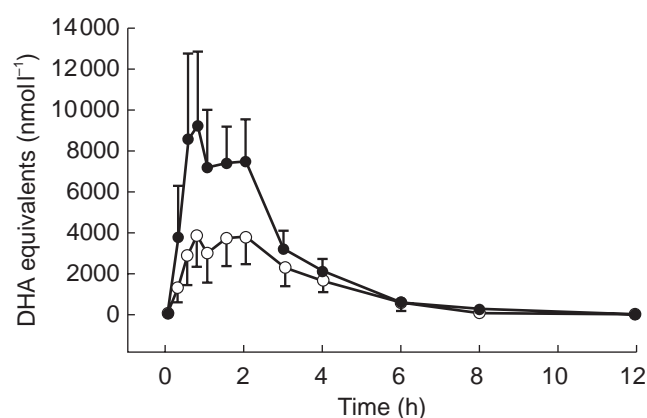
Figure 2 Mean antimalarial activity ($\pm$s.e. mean) in DHA equivalents for oral administration of artesunate, on the first ($\circ$) and second day ($\bullet$), during the first 12 h after drug administration.

The antimalarial C_{max} and AUC(0,∞) for both artesunate and artemether were non-significantly lower after dosing on the first day than after dosing on the second day (Table 2, Figure 2). The artemether antimalarial activity median apparent volume of distribution (V_z/F) was significantly smaller when the drug was administered on the second day than when given on the first day. The artesunate V_z/F did not significantly differ between the first and second days (Table 2). The median (range) antimalarial bioavailability of artemether relative to that of artesunate was significantly higher when artemether was given on the second day (72% (44–118)) than when given on the first day (31% (17–100)) ($P=0.018$) suggesting that acute malaria differentially affects the absorption and disposition of these drugs, but that during recovery these differences become less pronounced.

Table 2 Median (range) and mean (95% CI)[a–d] pharmacokinetic variables after oral administration of artesunate and artemether on the 1st and 2nd day. Bioassay in DHA equivalents, except molar dose. Abbreviations as in Table 1.

Variable	Artesunate			Artemether		
	Day 1	Day 2	P	Day 1	Day 2	P
Dose (nmol kg^{-1} body weight)	10 272 (10 026–10 518)	10 806 (10 085–11 527)[a]	0.2	13 922 (12 994–14 850)	13 234 (12 917–13 551)[b]	0.2
t_{lag} (h)	0.25 (0.25–0.25)	0.25 (0.25–0.25)	1.0	0.25 (0.25–0.25)	0.50 (0.25–1.50)	0.03
t_{max} (h)	2.00 (0.50–4.00)	1.13 (0.50–4.00)	0.5	1.0 (0.75–4.00)	2.00 (1.00–6.00)	0.2
C_{max} (nmol l^{-1})	5118 (3178–8359)	16 998 (4791–24 633)	0.08	3091 (909–4663)	3454 (1480–4339)	0.9
$t_{1/2}$ (h)	0.75 (0.49–2.30)	2.59 (0.55–4.71)	0.2	3.34 (1.30–5.44)	2.05 (1.22–4.32)	0.2
$AUC(0,\infty)$ (nmol l^{-1} h)	14 184 (10 336–18 032)	23 551 (15 764–31 338)[c]	0.06	9274 (6370–12 178)	12 984 (10 400–15 568)[d]	0.09
V_z/F (l kg^{-1})	0.78 (0.52–3.15)	1.94 (0.34–5.59)	0.7	9.46 (3.80–13.85)	2.84 (1.73–6.40)	0.017
CL/F (l kg^{-1} h^{-1})	0.72 (0.49–1.57)	0.43 (0.27–1.00)	0.2	1.72 (0.79–2.55)	1.08 (0.69–1.38)	0.1
MRT (h)	2.71 (1.52–5.11)	2.34 (1.18–4.39)	0.8	3.65 (0.58–6.31)	4.29 (2.96–6.89)	0.5

Difference between means (95% CI): [a]534 (145, 923); [b]688 (187, 1189) nmol kg^{-1} body weight; [c]9367 (4935–13799); [d]3710 (1726–5694) nmol l^{-1} h.

Discussion

Studies of Vietnamese and Thai adults with uncomplicated *P. falciparum* malaria, using well validated h.p.l.c. with u.v. detection and bioassay methods, respectively, gave similar artesunate pharmacokinetic parameters to those described here [10, 15]. The results of the present study with artemether are similar to those reported using the sensitive technique of h.p.l.c. with electrochemical detection in the reductive mode [12, 16, 22].

Comparison of artemether pharmacokinetics between patients with uncomplicated malaria and volunteers [14, 16, 23] suggests that AUC and C_{max} are higher in patients with malaria than in healthy individuals. This also occurs with oral artesunate [15] and probably results from reduced drug clearance and contraction in the apparent volume of distribution in disease. Artemether, arteether, artelinic acid and artesunate are readily hydrolysed to DHA, and except for artesunate, this is mediated predominantly by cytochrome P450 CYP3A4 [24]. The activities of these hepatic and intestinal wall enzyme subfamilies are reduced significantly by acute malaria [25–27]. Marked auto-induction of artemisinin metabolism has been described [28] and may also occur for artemether [22]. Whether this applies to artesunate and DHA metabolism is not known for certain [22, 29, 30]. Food is also a potential confounder as artemether bioavailability increases with food [29] and patients will resume eating as they recover from malaria.

The data presented here allow a direct comparison of the pharmacokinetics of the two main artemisinin derivatives. Antimalarial activity profiles after artesunate gave significantly higher C_{max} and AUC values, a smaller apparent volume of distribution and more rapid clearance resulting in a shorter half-life and mean residence time in comparison with those after artemether (Table 2, Figure 1).

Thus oral artesunate resulted in greater antimalarial activity bioavailability than oral artemether, even though the artesunate dose used in this study was 29% lower in molar terms. These differences could have resulted from more complete absorption of artesunate, or faster and more complete conversion of artesunate to DHA, than that of artemether. The mean absolute antimalarial bioavailability of oral artesunate is 61% [15] but cannot be calculated for artemether as there is no intravenous preparation. The biotransformation of oral artemether to the more active metabolite DHA, is less complete than the equivalent biotransformation of oral artesunate. As the parent compound is some two to three times less active as an antimalarial than DHA, this would dilute the antimalarial activity [16].

Comparison of antimalarial pharmacokinetic and pharmacodynamic properties, adverse effect profiles, cost, availability, and influence on malaria transmission and the evolution of drug resistance are needed to make the correct choice of the most suitable antimalarial for general use [31–34]. Oral artesunate and artemether, at equivalent total mg kg^{-1} body weight doses, result in similar times to parasite clearance and adverse effect profiles in patients with uncomplicated falciparum malaria at similar cost [8, 9, 35]. To experimental mammals, intramuscular artemether is significantly more neurotoxic than intramuscular artesunate, or the oral artemisinin derivatives [36–38]. As yet, there is no evidence for any neurotoxicity in man or *in vivo* resistance to artemisinin derivatives. However, the development of resistance may well occur if artemisinin derivatives are used alone, inappropriate drug combinations are chosen or if suboptimal doses are used [33, 34].

The principal pharmacokinetic parameter determining the antimalarial response to the artemisinin derivatives, which have very steep concentration-effect relationships,

may well be the time that blood concentrations exceed the minimum parasiticidal concentration (MPC), rather than the C_{max} or AUC [32]. For the data presented here, after 4 mg kg^{-1} of artesunate and artemether, anti-malarial activity would exceed the *in vitro* IC_{90} of local *P. falciparum* isolates [18] for a median (range) of 9 (6–12) and 11 (8–12) h, respectively ($P=0.062$). Thus, although the mean antimalarial activity AUC following artemether was 43% of that following artesunate and the mean C_{max} 59% that of artesunate (despite a higher molar dose), artemether and artesunate give equivalent *in vivo* parasite clearance and duration above the *in vitro* IC_{90} after drug administration. These findings are consistent with the hypothesis that it is the duration for which blood concentrations exceed the MPC which is the key parameter in determining antimalarial response. Artemether antimalarial activity was more susceptible to acute disease effects than that of artesunate. Artemether would be more likely than artesunate to result in a blood concentration that was not maximally effective, particularly if the dose was reduced. Artesunate provides greater antimalarial activity than artemether for the same molar dose and cost.

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